

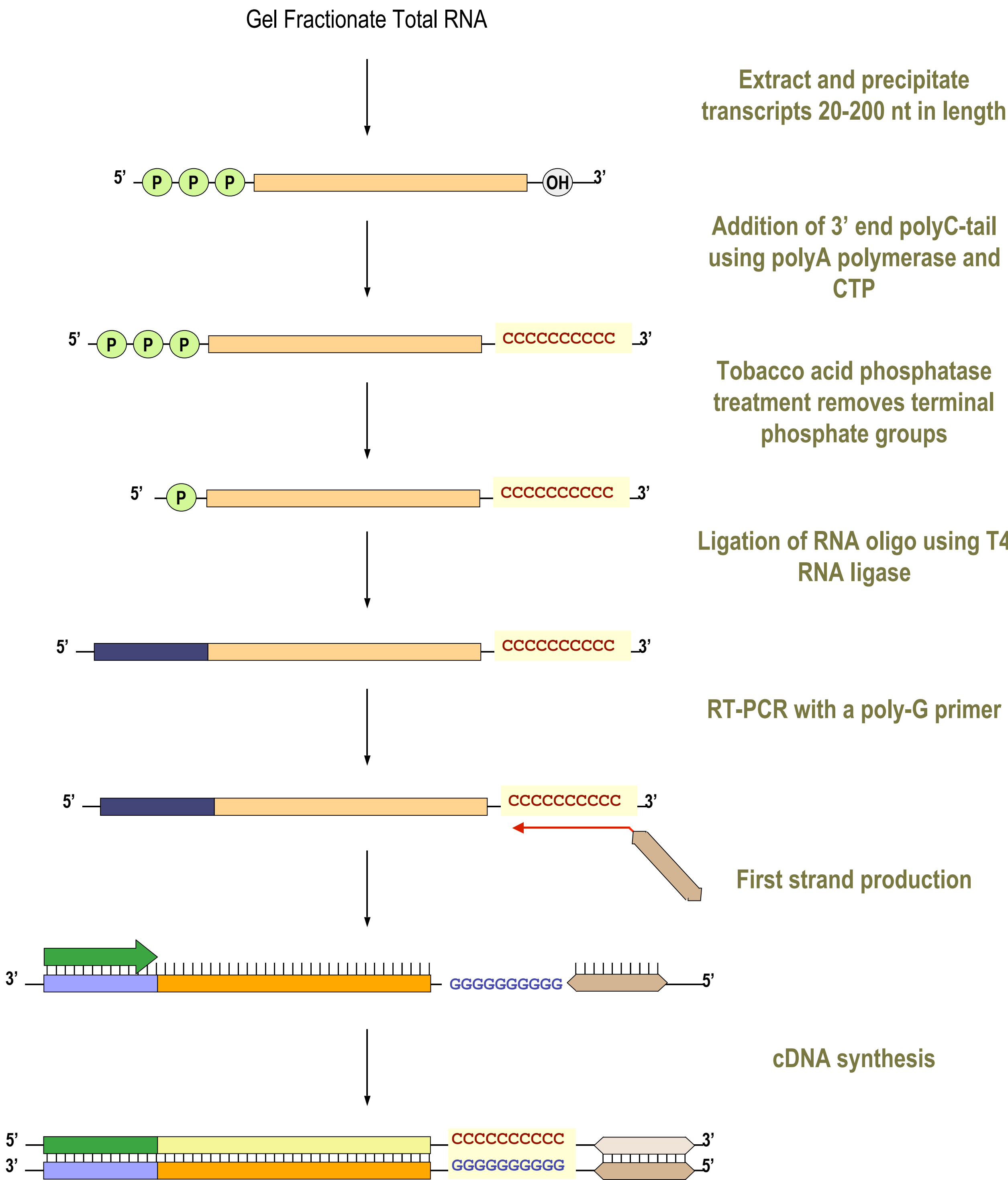
## ABSTRACT

Because one of the central aims of the 'Environmental Stress Pathway Project' is to elucidate regulatory networks critical to processes of interest to the DOE, the Computational Core has garnered valuable transcriptional and proteomic profiles under various environmental stress conditions. This data has been essential to enhancing our biological systems knowledge of the model metal reducer *Desulfovibrio vulgaris*. To further understand how this organism and its relatives compete in the environment and regulate their metabolism in contaminated sites, additional studies on intricate regulatory cascades are imperative. One potential alternative regulatory mechanism currently being targeted by the ESPP is that of small non-coding RNA molecules (sRNAs). Ranging in size from 20-200 nucleotides (nt), sRNAs predominantly affect gene regulation by binding to complementary mRNA in an anti-sense fashion and therefore provide an immediate regulatory response independent of protein modification. Whereas data are available for sRNAs in such prokaryotes as *Escherichia coli*, *Archaeoglobus fulgidus*, *Pseudomonas aeruginosa*, and *Vibrio* species, no information is available on metal-reducing bacteria, or for that matter, members of the delta-proteobacteria.

In an effort to identify sRNAs in *D. vulgaris*, a strategy for cloning total RNA ranging in size from 20-200 nt was employed. Following addition of directional aptamer sequences, cDNAs were produced and cloned for sequencing. Sequence analysis of a small portion of the resulting cDNA library yielded two identical ~65 nt sRNA clones (Dv-sRNA2) possessing complementary sequence to the RBS of open reading frame (ORF) DVU0678. While DVU0678 is adjacent to the Dv-sRNA2 gene, the ORF is transcribed from the opposite chromosomal strand. Northern analysis specialized for sRNAs verified the expression of Dv-sRNA2 as an individual transcript under anaerobic lactate/sulfate growth (LS4D medium). These data suggest that when Dv-sRNA2 is transcribed, translation of DVU0678 will be inhibited. DVU0678 has been annotated to encode a putative 34 amino acid protein unique to *D. vulgaris* strains Hildenborough and DP4, hampering our abilities to discern the role of DVU0678 in the cell. Further sequence analysis of the Dv-sRNA DNA locus by 'PromScan' identified a putative sigma<sup>54</sup>-recognition site (97% probability) 43 nt upstream of the predicted sRNA transcriptional start site and therefore suggests that Dv-sRNA2 may be member of the sigma<sup>54</sup> regulon. A perfect stem-loop terminator was also identified 26 nt downstream of the Dv-sRNA2 DNA sequence. Current analysis is underway to ascertain the expression profile for this sRNA as well as the effect over-expression has on the physiology and transcriptional response of *D. vulgaris* under multiple environmental conditions.

## MATERIALS AND METHODS

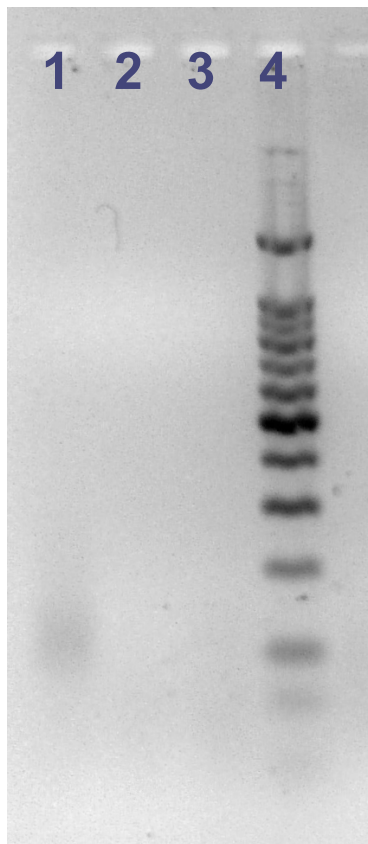
### Cloning of Small RNA Fraction



## RESULTS

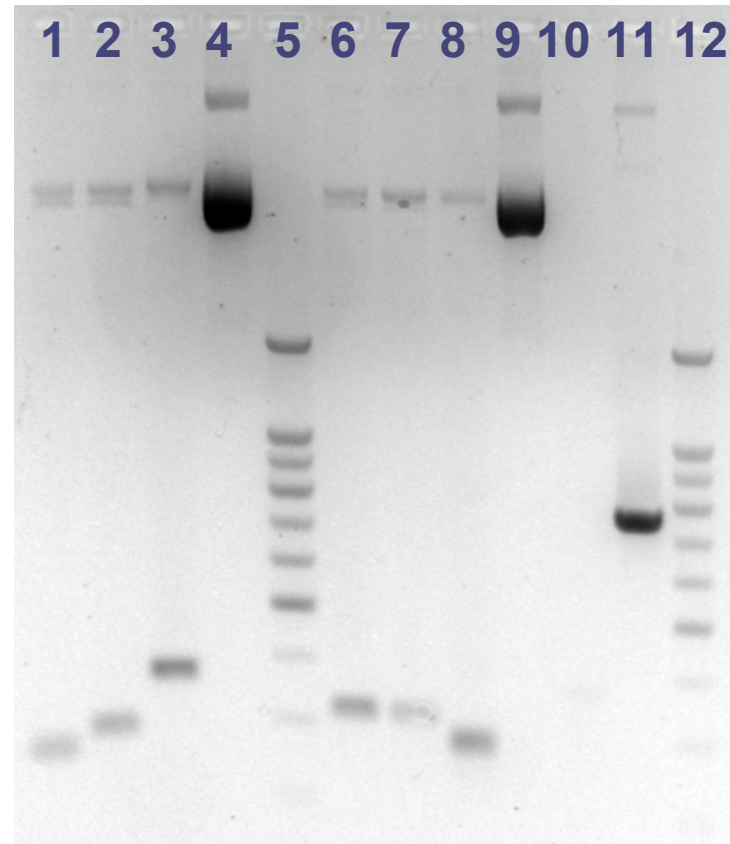
### Cloning and Sequencing of Small RNAs

#### cDNA for library



1: cDNA  
2: No template  
3: No reverse  
transcriptase  
4: 100 bp ladder

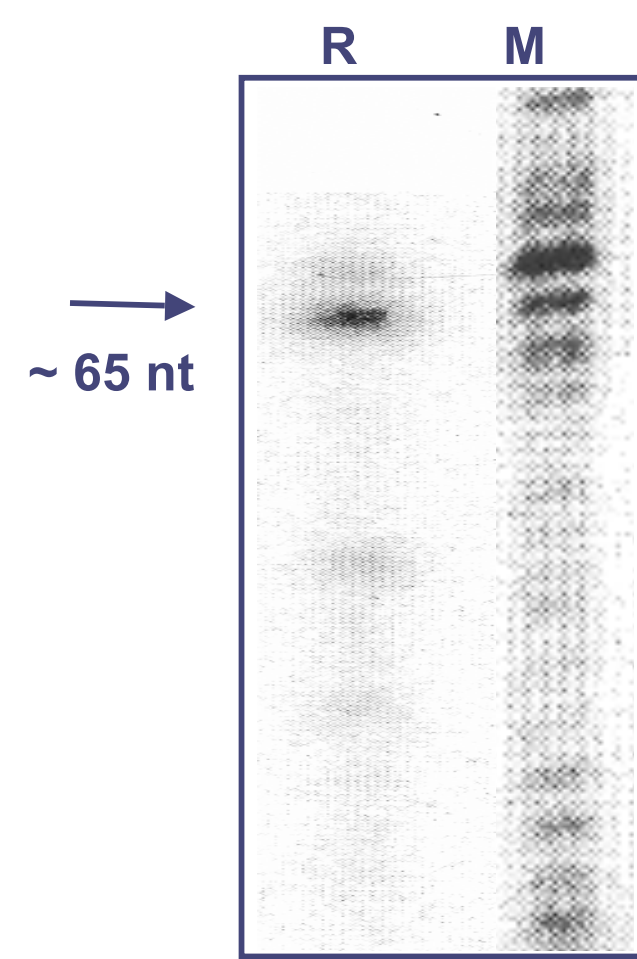
#### PCR verification of sRNA clone inserts



1-3: library clones  
4: plasmid only  
5: 100 bp ladder  
6-8: library clones  
9: plasmid only  
10: neg. control  
11: primer positive control  
12: 100 bp ladder

### Expression of Dv-sRNA2

#### Northern Verification of Dv-sRNA2 Expression

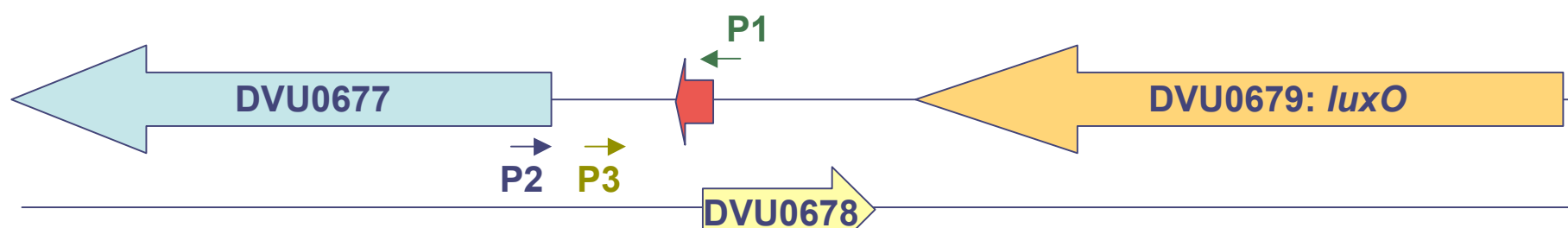
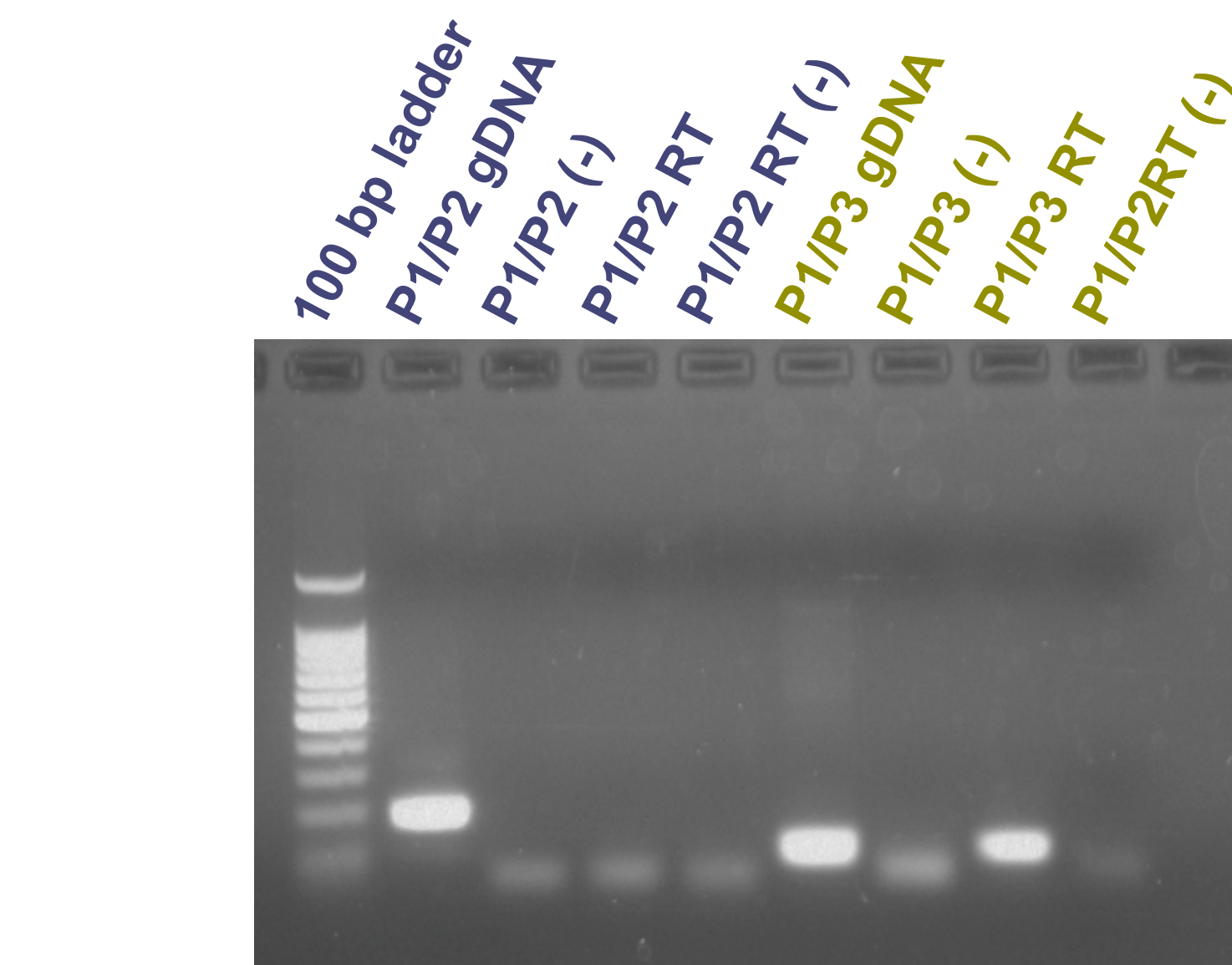


R: 10 µg total RNA  
M: Marker

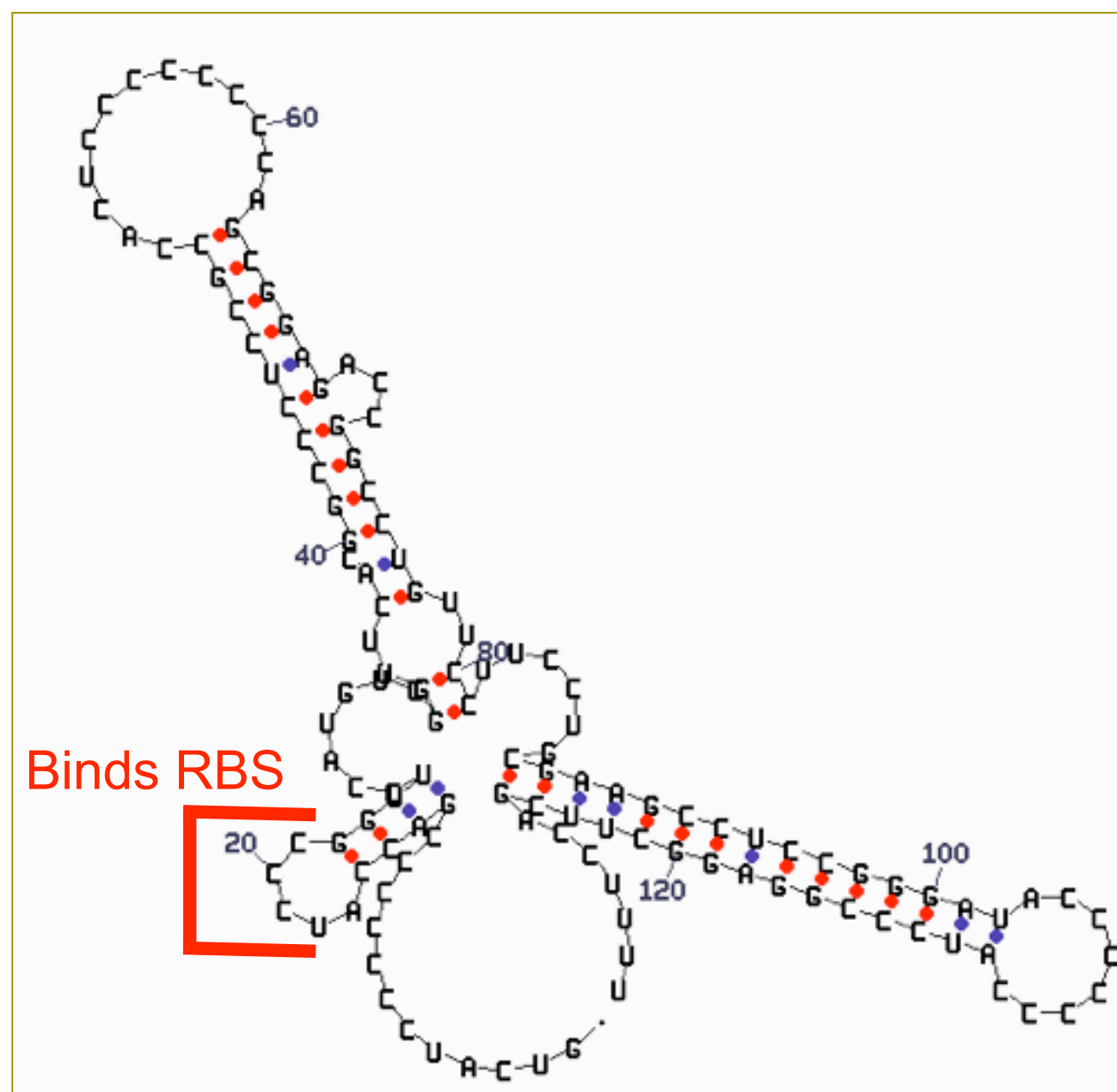
### Sequencing Results of Library Subsample

clone	size in nt	genes nearby	description
2A	65	DVU0677(-) transglycosylase DVU0678(+) putative protein DVU0679(-) LuxO	antisense to RBS of DVU0678; transcript starts 37 nt into the 5' end of DVU0678 and ends 24 nt into promoter region of DVU0678
4B	31	DVU0489(+) paak-1 DVU0490(-) signal transduction; frame shift DVU0491(+) HDIG domain protein	middle portion of the DVU0490 ORF, thus it is transcribed regardless of the frameshift
4C	135	DVU2401(-)hydrogenase DVU2402(-)hdrA	part of heterodisulfide reductase polycistronic transcript; transcript starts 113 nt into the 3' end of DVU2402 and ends 34 nt into 5' end of DVU2401
2-7	55	DVU3106(-) GGDEF domain protein DVU3107(+) cytochrome c DVU3108(-) Na/H anitporter NhaC	3' end of DVU3107 including terminator region
4-1	23	DVU0953(+) tyrosyl-tRNA DVU0954(-) organic solvent protein	transcript starts 18 nt after the 3' end of DVU0953; since on same strand probably the terminating region
4-2	35	VIMSS410439(+) tRNA-Gln VIMSS410440(+) tRNA-Glu	codes for the 5' end of tRNA-Glu (glutamic acid)
133	15	tRNA for Ile	transcript starts 37 nt upstream of the tRNA Ile message
136	110	23S rRNA	transcript for 5S rRNA
137	29	DVU2401(-)hydrogenase DVU2402(-)hdrA	part of heterodisulfide reductase polycistronic transcript; transcript starts 195 nt into the 5' half of DVU2401
144	60	DVU1305(+) ribosomal protein L23: rplW DVU1306(+) ribosomal protein L2: rplB DVU1307(+) ribosomal protein: rpsS	middle portion of the DVU1306 ORF; part of polycistronic ribosomal protein transcript

### RT-PCR Analysis of Dv-sRNA2



### MFOLD Secondary Structure Prediction of Dv-sRNA2



## SUMMARY

61 nt target starts 37 nt into  
the 5' end of DVU0678



DVU0678 message: 34 AA  
hypothetical protein

RBS of DVU0678 binding site  
blocked if **small RNA 2A**  
present: **No translation.**



## CONCLUSIONS

• In addition to computational searches, random cloning of the small RNA fraction can be used to identify unknown sRNAs.

• The cloning method does not discriminate between degraded mRNAs and non-coding RNAs.

• Dv-sRNA2 is a ~65 nt sRNA in *D. vulgaris* that is expressed under normal growth conditions.

• Dv-sRNA2 is anti-sense to the RBS of DVU0678, an ORF which encodes a putative protein.

• It is predicted that when Dv-sRNA2 is expressed, DVU0678 will not be translated based on the inability of the ribosome to bind.

• Sequence analysis identified a putative sigma<sup>54</sup>-binding site with 97% probability (PromScan) 43 bp upstream of the Dv-sRNA2 gene and a perfect rho-independent terminator 26 bp downstream.

• RT-PCR confirmed that Dv-sRNA2 is not part of a 5' untranslated region of the DVU0677 ORF.

• To ascertain the cellular role of Dv-sRNA2, current work is underway to over-express Dv-sRNA2 in *D. vulgaris*. Northern analysis under various stress conditions is also on-going to determine the expression pattern of Dv-sRNA2.

## ACKNOWLEDGEMENT

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